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A prospective study of high-risk commercial sex workers in Senegal has shown that HIV-2 infection may reduce the risk of subsequent HIV-1 infection; these findings have been confirmed and extended, now with 13 years of observation. While exploring the biological mechanisms behind this natural protection, we found that a significant proportion of peripheral blood mononuclear cells obtained from HIV-2-infected subjects resisted *in vitro* challenge with CCR5-dependent HIV-1 viruses but not CXCR4-dependent viruses. High levels of β-chemokines, the natural ligands of the CCR5 coreceptor, were correlated with low levels of viral replication, and resistance was abrogated by antibodies to β-chemokines. Our results suggest that β-chemokine-mediated resistance may be an important correlate of HIV protection against HIV-1 infection and relevant to HIV vaccine design.

HIV-2 has ≈50% genetic homology to the prototype AIDS virus, HIV-1. Despite structural and functional similarities, transmission is less efficient, and progression to AIDS is significantly slower for HIV-2 than HIV-1 (1, 2). Based on these attenuated properties, it was hypothesized that HIV-2 might confer some protection against the more virulent HIV-1. Indeed, epidemiological analysis of HIV-1 incidence rates in a large cohort study of female sex workers who were exposed to both HIV-1 and HIV-2 revealed that the risk for HIV-1 infection in individuals infected with HIV-2 was reduced dramatically (3). Follow up of this cohort has continued to demonstrate HIV-1 protection, ranging from 52% to 74%, depending on the study design (4, 5).

Few studies have investigated the mechanisms by which HIV-2 infection might alter the natural course of HIV-1 infection. *In vitro* studies have demonstrated that HIV-2 can inhibit HIV-1 replication by interfering with an unidentified molecular pathway at the intracellular level (6, 7). Recently, it was shown that the TAR region from the HIV type 2 (HIV-2) could suppress HIV-1 transcription and replication and that HIV-2 RNA could therefore act as an inhibitor of HIV-1 in coinfected cells (8). Receptor-mediated viral interference would be another mechanism whereby both viruses compete for the same target cell receptor, and infection with one virus might down-regulate the receptor preventing superinfection (9). However, the small fraction of HIV-infected cells *in vivo* would seem to preclude the feasibility of such mechanisms. Robust and cross-reactive humoral and cellular immunity conferred by HIV-2 infection in people has been documented and may also play an important role in altering susceptibility to subsequent HIV-1 infection (10–12).

To identify mechanisms of HIV protection further, we evaluated the susceptibility of HIV-2-infected peripheral blood mononuclear cells (PBMCs) to HIV-1 challenge *in vitro.* The relative susceptibility of PBMCs to HIV infection has been used to identify and/or verify inherent or acquired *in vivo* resistance in exposed uninfected individuals (13–15) and clinical progression in patients infected with HIV-1 (16) and has also been used as a potential marker of vaccine efficacy (15). The *in vitro* assay allowed us to manipulate and evaluate the specific contribution of various cell subpopulations in response to virus exposure and infection.

Methods

Study Subjects. Our study subjects are members of the previously described cohort of registered commercial sex workers in Dakar, Senegal (1, 2). Study participants were enrolled after receiving informed consent, and we obtained peripheral blood samples from 28 asymptomatic HIV-2-infected and 12 HIV-negative individuals. Seven normal U.S. blood donors were also included in the study as controls for cell manipulation and storage conditions. CD4 counts determined by fluorescence-activated cell sorter analysis (Facscount, Becton Dickinson) and years of positivity are provided in Table 1. Serostatus was defined by HIV-1 and HIV-2 immunoblot and confirmed with recombinant envelope peptides and HIV-specific PCR (17, 18).

Cell Separation. Blood was collected on EDTA. PBMCs were obtained by centrifugation on lymphocyte separation medium (ICN) and subsequently cryopreserved in 90% (vol/vol) FCS/10% (vol/vol) DMSO.

Infection. For each experiment, cells were stimulated for 48 h with 5 μg/ml phytohemagglutinin (PHA) in complete IL-2 medium [RPMI medium 1640 supplemented with 20% (vol/vol) FBS, 1% antibiotics, and 100 units/ml IL-2]. PHA was then removed, and the cells were counted. Cell viability was evaluated by trypan blue exclusion dye, and cultures were set up in 96-well microplates at a density of 250,000 cells per 200 μl of complete IL-2 medium. All viral stocks were propagated in PBMCs from normal donors, harvested at the peak of infection, and stored aliquoted at −75°C. Infectivity titers were determined simultaneously for all viruses in the present study with serial 4-fold dilutions in PBMCs from one donor. Cell cultures were inoculated with 600 tissue culture 50% infective dose ($TCID_{50}$) of each virus, marking day 0 of the experiment. On day 2, cells were washed with 50 times their volume of complete medium. Thereafter, cell cultures were sampled and fed with 50% replacement of the total culture volume at days 4, 7, 10, 14, and 18, unless otherwise indicated. HIV-1 replication was assessed by p24 ELISA in culture supernatants (NEN). When sufficient cells were available, the viral challenge experiments were performed in duplicate.

β-Chemokines. Cells were stimulated with PHA for 2 days and placed in culture at a density of 1×10^6 cells per ml. β-Chemokine levels were determined in culture supernatants by ELISA (R & D Systems) at day 4 of the experiment.

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Abbreviations: PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; $TCID_{50}$, tissue culture 50% infective dose.

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Table 1. HIV-1 JR-CSF infectability of PBMCs from HIV-2-infected individuals

Patient identification no.	Peak p24, pg/ml	Positive years	CD4+	Phenotype
2602	10	4	744	R
2	13	5	483	R
1082	33	9	184	R
31	58	7	458	R
2269	131	5	689	R
342	230	11	603	R
1704	374	6	1,027	R
879	571	9	ND	R
636	619	5	1,285	R
1503	830	5	1,089	R
1972	1,520	<1	1,182	R
2712	1,936	5	577	R
1079	3,462	3	1,030	R
82	4,889	11	1,647	R
22	9,984	8	899	S
337	35,883	6	522	S
912	49,032	11	671	S
2378	55,600	4	1,162	S
2028	60,117	9	1,475	S
1960	85,200	6	728	S
2822	101,911	4	ND	S
3199	103,439	2	ND	S
996	122,065	7	611	S
1814	146,433	10	1,095	S
2661	176,815	5	1,198	S
524	198,968	11	1,451	S
288	643,919	9	905	S
1130	647,340	8	854	S

Positive years, difference between sample year and infection date (when known) or date of the first positive bleed. CD4+, CD4+ cell counts within 1 year of sample collection. ND, not determined; R, resistance (peak p24 levels <5,000 pg/ml); S, susceptibility.

CD8 Depletion. CD8 depletion (>90%) was performed with the use of antibody-coated MACS microbeads (Miltenyi Biotec, Auburn CA) followed by magnetic separation according to the manufacturer's instructions.

Antibody Neutralization of β-Chemokines. A mixture of polyclonal affinity-purified antibodies against β-chemokines at a concentration of 50 μg/ml each or control goat Ig at a concentration of 150 μg/ml (R & D Systems) was supplied at the time of viral challenge and at each subsequent replacement of culture medium during the 18 days of the experiment. Viral replication kinetics, after challenge of 250,000 resistant PBMCs derived from individuals infected with HIV-2 ($n = 6$) with 600 $TCID_{50}$ of HIV-1JR-CSF, were observed in the presence of β-chemokine-neutralizing antibodies or control antibody.

Statistics. Statistical analysis was performed with STATA (version 4.0, Stata, College Station, TX).

Results

The initial screening for resistance among the HIV-2-infected PBMCs was performed by challenge with a macrophage-tropic HIV-1 strain (JR-CSF), considered to be representative of NSI strains implicated in heterosexual transmission (19, 20). HIV-1JR-CSF uses the CCR5 as a major coreceptor for entry and has been used in various studies to screen for resistance among exposed uninfected individuals and HIV-1-infected long-term nonprogressors (13, 21, 22). PBMCs from all 19 HIV-negative controls were found to be readily infectable by HIV-1JR-CSF, although variable in their peak levels of infection. However, a clear difference in infectivity was observed when the HIV-2-infected PBMCs were challenged similarly with HIV-1JR-CSF. Cultures with a p24 antigen level of 5,000 pg/ml or lower at day 10 were classified as resistant cultures in the HIV-2-infected PBMCs. This cut-off value also represented 98% inhibition of the median value obtained from controls. Of 28 (50%) HIV-2 PBMCs, 14 exhibited this resistant phenotype compared with none of the 19 HIV negative PBMCs (Fisher exact test, $P = 0.002$; Table 1). Viral replication kinetics indicated that resistant and susceptible cultures had reached a plateau of infection during the 18-day period of observation (Table 1 and Fig. 1).

When the same PBMCs were challenged with a 600 $TCID_{50}$ viral inoculum of HIV-1IIIB, a CXCR4-dependent (X4) virus, the results were strikingly different. Of 28 cases of HIV-2-infected PBMCs, 26 supported replication of this T tropic virus just as well as the control PBMCs (Fig. 1). The remaining two cases exhibited peak p24 levels more than 1 log higher with HIV-1IIIB than with HIV-1JR-CSF. As such, HIV-1IIIB replicated freely, unlike HIV-1JR-CSF which seemed to be inhibited by HIV-2 infection. The infectability of the PBMCs, as demonstrated by their susceptibility to the T tropic X4 virus, showed that the intrinsic ability of the PBMCs to support viral replication remained intact and that resistance was probably not associated with CD4, the primary cellular receptor for viral entry.

Because target cell viability is critical to viral replication, we repeatedly evaluated parallel uninfected cultures for cell count and cell viability over time. We were unable to demonstrate any consistent differences in cell numbers or viability between HIV-2-infected and HIV-negative PBMCs or between resistant and susceptible cells within the group of HIV-2-infected samples (data not shown). Furthermore, the ability of all HIV-2 PBMCs to support HIV-1IIIB virus infection suggested that resistance was not due to cell viability differences. Because *ex vivo* production of endogenous HIV-2 might interfere with the infection of a related retrovirus, parallel noninoculated cultures of the HIV-2 PBMCs were assayed over time for endogenous HIV-2 production with a simian immunodeficiency virus core antigen ELISA (Coulter). However, no detectable levels of p26 were found. The potency of the resistance was evaluated with 10-fold higher inoculum doses of HIV-1, and another CCR5-NSI virus was also evaluated in limited studies with essentially the same results (data not shown). In addition, we recently described in nine other individuals infected with HIV-2 similar observations with two different isolates: X4/R5 isolate HIV-1MN and R5 isolate HIV-1BAL (15).

We also sought to evaluate characteristics of the study subjects that might be associated with *in vitro* resistance. CD4+ cell counts determined within 1 year of the PBMC collection did not seem to correlate with the resistance phenotype (Table 1). Similarly, the minimum time infected, defined by the difference between sample date and seroconversion date or first sample date in the case of prevalent individuals, did not differ between the two groups (Table 1). Thus, it seemed that the observed HIV-1 resistance was specific to CCR5-dependent (R5) viruses and that this resistance was not due to altered cell viability, endogenous HIV-2 production, or definable characteristics of the individuals infected with HIV-2.

The two most widely used coreceptors for HIV-1 are CCR5 and CXCR4, expressed by both activated T lymphocytes and mononuclear phagocytes. CCR5 is the natural receptor for the β-chemokines RANTES, MIP-1α, and MIP-1β and also serves as the HIV coreceptor preferentially used by NSI strains of HIV-1 and HIV-2 (23–28). CXCR4, a receptor for the CXC chemokine SDF-1, is used by MT2 tropic or SI strains of HIV-1 and some HIV-2 strains (29), although HIV-2 is considered to

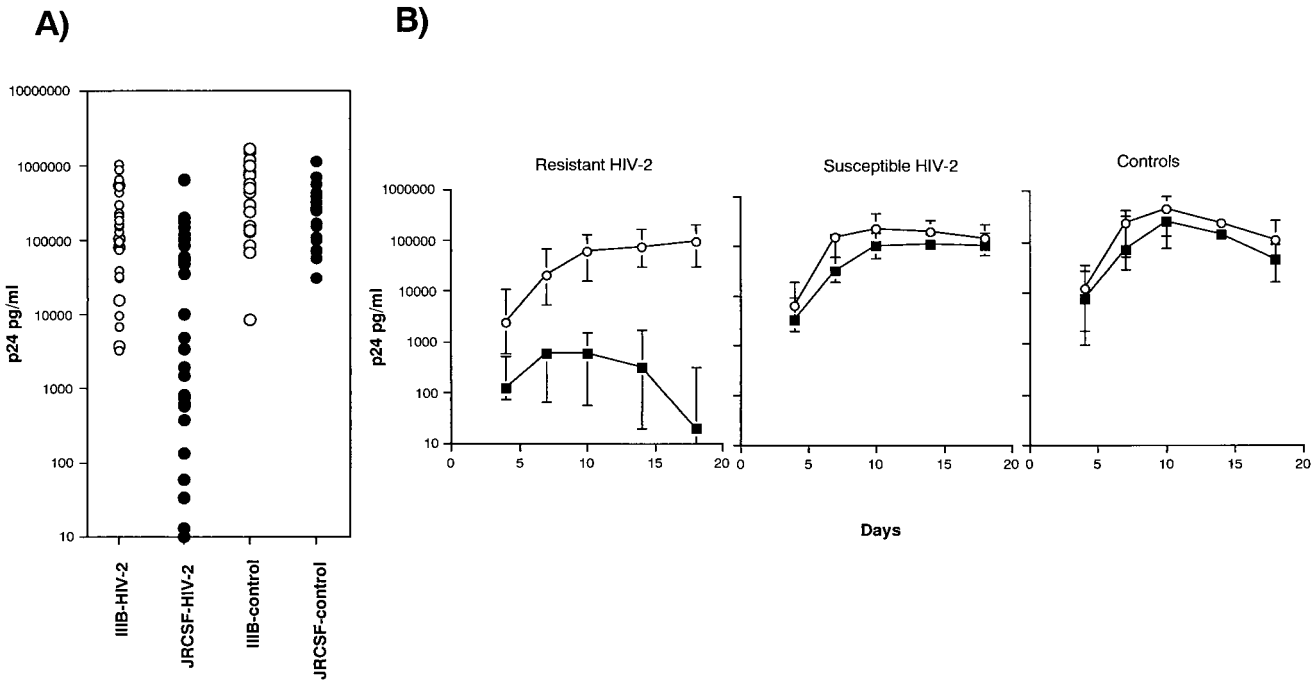

Fig. 1. *In vitro* infectability of PBMCs from individuals infected with HIV-2 or controls by different strains of HIV-1. (*A*) PBMCs from individuals infected with HIV-2 ($n = 28$) or controls ($n = 19$) were challenged with 600 $TCID_{50}$ of HIV-1JR-CSF or HIV-1IIIB and monitored for p24 production over a period of 18 days. Values presented correspond to p24 levels at day 10. HIV-1JR-CSF infection levels in HIV-2-infected PBMCs were significantly lower than those with HIV-1IIIB (Wilcoxon rank-sum test, $P = 0.0001$). (*B*) Replication kinetics of HIV-1JR-CSF (filled squares) and HIV-1IIIB (open circles) in PBMCs from individuals infected with HIV-2 that were found to be resistant to HIV-1JR-CSF (*Left*; $n = 14$) or susceptible (*Center*; $n = 14$) and from negative controls (*Right*; $n = 19$). The median values and the first and third quartiles are plotted.

be more promiscuous in its coreceptor usage (27, 28, 30–32). In 1995, Cocchi and colleagues (33) described suppression of HIV replication by CD8+ T cells from individuals infected with HIV mediated by the β-chemokines RANTES, MIP-1α, and MIP-1β; potent and specific suppression of various HIV-1, HIV-2, and simian immunodeficiency virus strains was mediated by blockage of the critical viral coreceptors.

In our study, it seemed that HIV-2-associated resistance to HIV-1 depended on the strain of HIV-1, preferentially associated with R5 as opposed to X4 strains. Therefore, we suspected that resistance was mediated via blocking of the R5 coreceptors by their natural ligands, the β-chemokines (23–28). Levels of RANTES, MIP-1α, and MIP-1β were determined after PHA stimulation in parallel uninfected cultures of the same HIV-2-

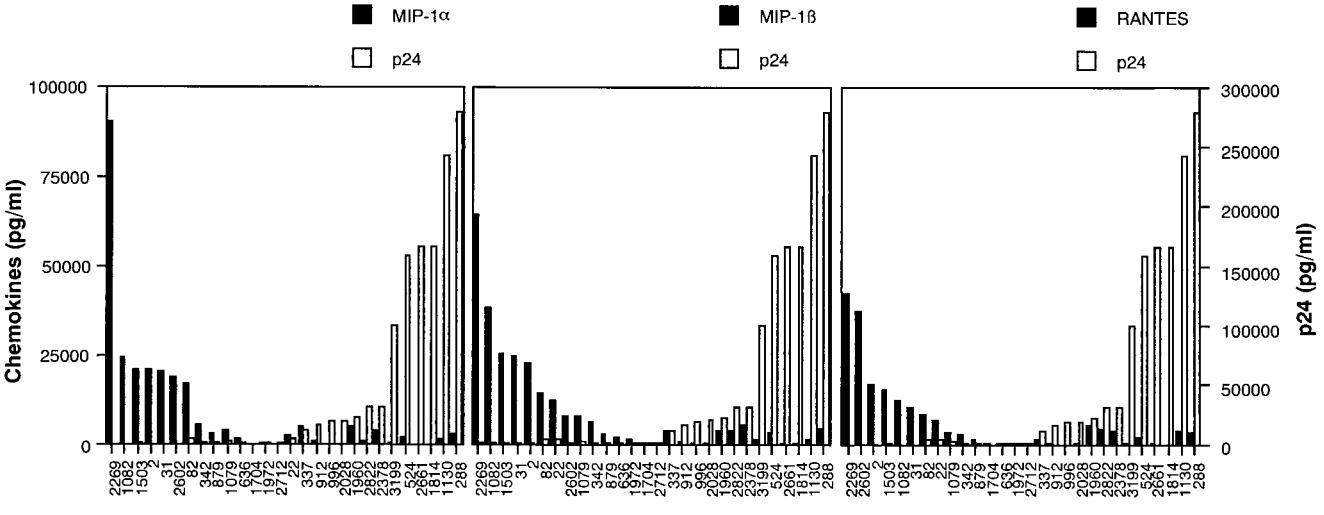

Fig. 2. β-Chemokine production inversely correlates with resistance of PBMCs to HIV-1JR-CSF infection. MIP-1α, MIP-1β, and RANTES production *in vitro* by PBMCs was inversely correlated with the ability of the same cells to support replication of HIV-1JR-CSF as determined by p24 levels (dark bars) at day 7 after viral challenge. There was an inverse correlation between β-chemokine and p24 levels [Spearman's correlation test for MIP-1α ($P = 0.009$), for MIP-1β ($P = 0.03$), and for RANTES ($P = 0.04$)].

infected PBMCs that were challenged with HIV-1JR-CSF. We were able to demonstrate a strong inverse correlation between all three β-chemokines measured at day 4 and p24 production in culture supernatants infected with HIV-1JR-CSF at days 7 and 10 of the experiment (Fig. 2). Most resistant PBMCs derived from individuals infected with HIV-2 clustered together as high β-chemokine producers, whereas susceptible PBMCs produced relatively less β-chemokines.

Having demonstrated that PBMCs derived from individuals infected with HIV-2 secreted increased levels of β-chemokines after stimulation with PHA, we sought to delineate and characterize further the role of β-chemokines in mediating HIV-1 resistance observed in our system. Although the β-chemokines might be the major suppressors of M tropic virus replication, other immune mechanisms or cellular factors may also affect the replication of M tropic viruses, accounting for the resistance we identified. For example, a 32-bp deletion in the CCR5 coreceptor has been associated with resistance to infection by macrophage tropic strains of HIV-1 (13, 34). In the African populations studied, this mutation is relatively rare, and we have confirmed this rarity independently by PCR genotypic analysis of the CCR5 receptor in our Senegalese study population (35). Thus, excess β-chemokine production from HIV-2-infected PBMCs did not seem to be due to a mutant CCR5 receptor.

Several studies have shown that both soluble and cell contact-dependent factors contribute to the HIV suppressive effects of CD8+ T cells (21, 22, 36, 37). Because CD8+ cells were considered to be a significant source of β-chemokines (13, 14, 33), it was conceivable that their depletion from the resistant HIV-2-infected PBMCs might counteract the resistance to HIV-1JR-CSF. Indeed, we observed a remarkable increase in p24 production after the removal of CD8+ cells from five HIV-2-infected PBMC cultures (Fig. 3*A*). However, the depletion of CD8+ cells did not return the cells to 100% susceptibility (data not shown). Within the PBMC cell population, cells other than CD8+ cells may be capable of producing β-chemokines, including both monocytes and natural killer cells (38–40). β-Chemokines also seem to be expressed as part of the cell-mediated response to HIV infection including the generation of proliferative and cytotoxic responses. After antigen-specific activation of CD8+ cytotoxic lymphocytes, β-chemokines bound to sulfated proteoglycans are secreted from cytolytic granules with granzyme A. Both granzyme A and β-chemokines are capable of inhibiting HIV-1 infection (21, 22), thereby enabling CD8+ cells to exert an antiviral effect through lysis of infected cells as well as inhibition of free virus (21, 22).

We subsequently followed two different experimental approaches to clarify further the role of β-chemokines in the observed resistance to HIV-1JR-CSF infection. First, we hypothesized that if the resistance was mediated by β-chemokines or another soluble factor, then we could provide them to otherwise susceptible PBMCs and expect the cells to exhibit a resistant phenotype. Indeed, when previously identified resistant PBMCs from individuals infected with HIV-2 were cultured in the upper chamber of a trans-well system, the cell-free supernatant significantly reduced the ability of normal susceptible donor PBMCs to support replication of HIV-1JR-CSF (data not shown). Although this experiment did not unambiguously demonstrate that β-chemokines were the sole factors involved, it confirmed the soluble cell-free nature of the resistance.

Second, to establish further the central role of β-chemokines in HIV-1 resistance, we evaluated the cell susceptibility on antibody-mediated neutralization of the β-chemokine activity. Culture media were continuously supplied with a mixture of polyclonal neutralizing antibodies to RANTES, MIP-1α, and MIP-1β or control antibody, starting at the time of HIV-1JR-CSF inoculation, and the kinetics of p24 accumulation were assessed over time. Neutralization of β-chemokines had a dramatic effect on viral replication. In six of six previously resistant HIV-2 PBMCs, there was a significant increase in HIV-1JR-CSF infection levels with up to a 3-log increase in p24 levels (Fig. 3*B*). No significant effect was observed in two control PBMC cultures where HIV-1JR-CSF replication was already vigorous. Although it was not possible to assess individually the contribution of each β-chemokine to resistance caused by sample and cell number restrictions, it is known from previous studies that the overall activity of β-chemokines is synergistic (21, 22, 33, 38–40). The peak p24 levels that were achieved in some HIV-2-infected PBMC cultures after β-chemokine neutralization were still lower

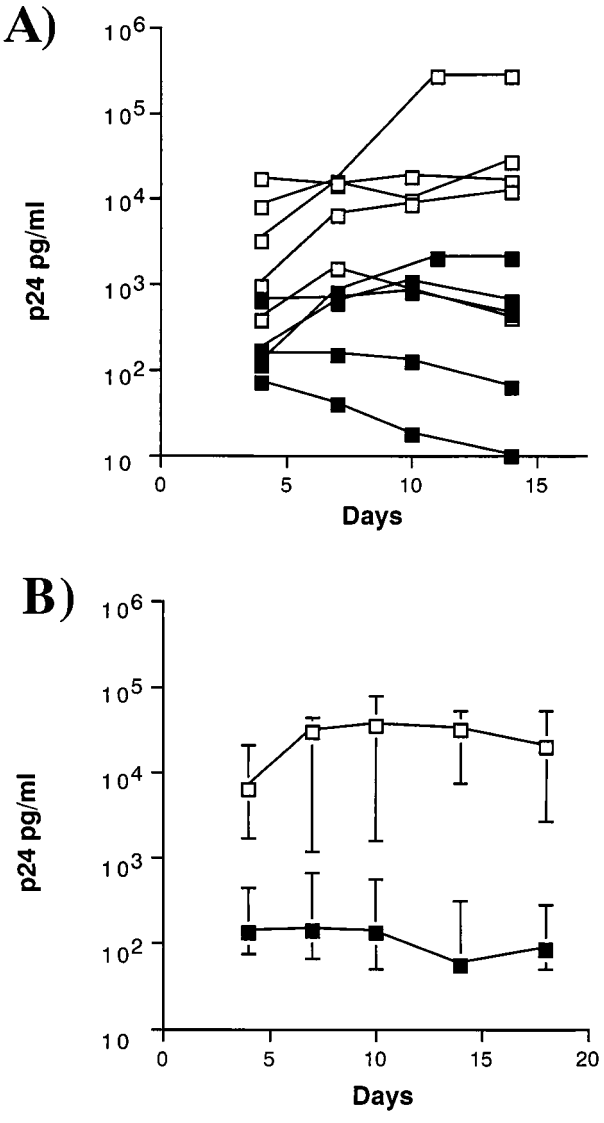

Fig. 3. Role of β-chemokines in HIV-1JR-CSF resistance *in vitro* exhibited by HIV-2 PBMCs. (*A*) PBMCs (filled squares) and the CD8-depleted fraction (open squares) derived from five individuals infected with HIV-2 were challenged with 600 $TCID_{50}$ of HIV-1JR-CSF, and p24 production was monitored for 14 days. (*B*) Antibody neutralization of RANTES, MIP-1α, and MIP-1β abrogated the HIV-2-associated resistance to R5 HIV-1 virus. Viral replication kinetics, after challenge of 250,000 resistant PBMCs derived from individuals infected with HIV-2 ($n = 6$) with 600 $TCID_{50}$ of HIV-1JR-CSF, were observed in the presence of β-chemokine-neutralizing antibodies (open diamonds) or control antibody (filled squares); median and first and third quartiles are shown. For clarity of presentation, p24 levels <50 pg/ml were assigned a value of 50 pg/ml.

than those obtained from control PBMCs. It is likely that the residual resistance could be attributed to incomplete β-chemokine neutralization and/or the presence of non-β-chemokine mediators. Our results would also be compatible with the presence and activity of as-yet unidentified β-chemokine(s) that our antibody treatment could not neutralize. It has been reported recently that certain viruses such as cytomegalovirus and human herpesvirus-8 encode chemokine-like molecules (41, 42). Because HIV-2 infection might render the host more susceptible to such pathogens, this scenario represents an intriguing possible explanation of our results. In two resistant HIV-2 PBMCs, β-chemokine antibodies were added to the cell culture, either at the initial viral challenge and at each subsequent feeding or once at the time of challenge, resulting in similar effects on viral replication (data not shown). This experiment implies that the effect of β-chemokines is exerted at viral entry and that the cells that are infected initially are those responsible for the majority of p24 production.

Discussion

The elucidation of the potential benefit or selective advantage that HIV-2 might gain by promoting the overproduction of β-chemokines in host cells would help to explain the phenomenon of resistance that we describe in the present study. The coreceptor requirements for HIV-2 or simian immunodeficiency virus are not as stringent as with HIV-1, and redundancy is common (27, 28, 30–32). One plausible explanation suggests that the coreceptor promiscuity of HIV-2 is an adaptive and selectable change whereby HIV-2 would be less susceptible to the CCR5 blockade as mediated by excess β-chemokines. In this way, β-chemokines would disproportionately affect HIV-1 and have little control over HIV-2 infection. Alternatively, β-chemokine overproduction could have an effect on the *in vivo* HIV-2 dynamics where significantly lower plasma viral loads are independent of sufficient proviral templates, consistent with β-chemokine neutralization (43).

Although a significant proportion of individuals infected with HIV-2 exhibited reduced susceptibility to HIV-1 challenge, the parameters by which the resistant phenotype would be induced in some individuals infected with HIV-2 and not in others remain unclear. One possibility could be differences in viral load or immune activation of the host. Alternatively, a specific HIV-2 strain or particular signature sequences might trigger the overproduction of β-chemokines. Sekigawa and colleagues (44, 45) have demonstrated a direct interaction of the HIV-2 envelope with the α-chain of the CD8 molecule and stimulation of β-chemokines in contrast to the gp120 of HIV-1. Their studies suggest that HIV-2 may possess a virus-specific mechanism for inducing β-chemokines that would protect from subsequent CCR5-mediated virus infection (44, 45).

Multiple lines of evidence have emerged to support the role of β-chemokines in HIV-1 resistance among PBMCs from individuals infected with HIV-2. These include the dichotomous resistance of R5 vs. X4 viral strains, increased levels of β-chemokines in cultures associated with low p24 levels, and the abrogation of resistance by neutralizing antibodies to β-chemokines. The involvement of β-chemokine-related mechanisms in resistance to HIV-1 infection, aside from genetic polymorphism in the CCR5 coreceptor (13, 33), has also been described in previous studies. T cell clones from exposed, uninfected partners of individuals infected with HIV-1 secreted high levels of β-chemokines on challenge with HIV-1-specific peptides (14). Hemophiliacs that received HIV-1-contaminated blood products and resisted infection were found to produce high levels of β-chemokines *in vitro* (46). A role for β-chemokines has been proposed in mechanisms of disease attenuation as well. In a recent study, specific HIV-1 proteins prompted ample *in vitro* production of β-chemokines in an HIV-1-infected hemophiliac long-term nonprogressor (47). Lehner *et al.* (48) described the successful immunization of macaques with a simian immunodeficiency virus *env*- and *core*-based vaccine that targeted the mucosa. Resistance in the vaccinated monkeys and in one of the control animals was associated with increased production of β-chemokines. It is therefore intriguing to consider antiretroviral vaccine strategies that might incorporate β-chemokine induction or coreceptor-blocking activity where the HIV-2 envelope might be of use.

In summary, *in vitro* observations from this study have elucidated an important biochemical mechanism implicated in the protection of individuals infected with HIV-2 from subsequent HIV-1 infection. Using an *in vitro* challenge system, we were able to demonstrate that a significant percentage (50%) of PBMCs derived from HIV-2-infected asymptomatic commercial sex workers could not support efficient replication of an R5 HIV-1 virus. It is of note that these figures are consistent with the epidemiological analysis, in which 52–74% of women infected with HIV-2 were found to be resistant to HIV-1 (3–5). Thus, the present study has described at least one potential mechanism by which HIV-2 alters susceptibility to HIV-1. It is certainly possible that other immune mechanisms are operative *in vivo* and may also contribute to the protection that has been observed in people. Nonetheless, these results suggest that β-chemokines may be important correlates of HIV-1 protection as exemplified by the documented protection demonstrated in people. These results implicate a unique mechanism that is not only viral suppressive but that may be readily adapted for immunoprophylaxis.

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1. Kanki, P. J., Travers, K. U., Mboup, S., Hsieh, C. C., Marlink, R. G., Guèye-Ndiaye, A., Siby, T., Thior, I., Hernandez-Avila, M., Sankalé, J. L., *et al*. (1994) *Lancet* **343,** 943–946.
2. Marlink, R., Kanki, P., Thior, I., Travers, K., Eisen, G., Siby, T., Traore, I., Hsieh, C. C., Dia, M. C., Guèye, E. H., *et al*. (1994) *Science* **265,** 1587–1590.
3. Travers, K., Mboup, S., Marlink, R., Guèye-Ndiaye, A., Siby, T., Thior, I., Traore, I., Dieng Sarr, A., Sankalé, J. L., Mullins, C., *et al.* (1995) *Science* **268,** 1612–1615.
4. Travers, K. U., Eisen, G. E., Marlink, R. G., Essex, M. E., Hsieh, C. C., Mboup, S. & Kanki, P. J. (1998) *AIDS* **12,** 224–225.
5. Kanki, P., Eisen, G., Travers, K. U., Marlink, R. G., Essex, M. E., Hsieh, C. C. & Mboup, S. (1996) *Science* **272,** 1959–1960.
6. Arya, S. & Gallo, R. C. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 4486–4491.
7. Rappaport, J., Arya, S. K., Richardson, M. W., Baier-Bitterlich, G. & Klotman, P. E. (1995) *J. Mol. Med.* **73,** 583–589.
8. Browning, C. M., Cagnon, L., Good, P. D., Rossi, J., Engelke, D. R. & Markovitz, D. M. (1999) *J. Virol.* **73,** 5191–5195.
9. Martin, R. A. & Nayak, D. P. (1996) *Virus Res.* **45,** 135–145.
10. Weiss, R. A., Clapham, P. R., Weber, J. N., Whitby, D., Tedder, R. S., O'Connor, T., Chamaret, S. & Montagnier, L. (1988) *AIDS* **2,** 95–100.
11. Fenyo, E. M. & Putkonen, P. (1996) *Immunol. Lett.* **51,** 95–99.
12. Bertoletti, A., Cham, F., McAdam, S., Rostron, T., Rowland-Jones, S., Sabally, S., Corrah, T., Ariyoshi, K. & Whittle, H. (1998) *J. Virol.* **72,** 2439–2448.
13. Paxton, W. A., Martin, S. R., Tse, D., Obrien, T. R., Skurnick, J., Vandevanter, N. L., Padian, N., Braun, J. F., Kotler, D. P., Wolinsky, S. M., *et al*. (1996) *Nat. Med.* **2,** 412–417.
14. Furci, L., Scarlatti, G., Burastero, S., Tambussi, G., Colognesi, C., Quillent, C., Longhi, R., Loverro, P., Borgonovo, B., Gaffi, D., *et al*. (1997) *J. Exp. Med*. **186,** 455–460.

15. Castillo, R. C., Arango-Jaramillo, S., John, R., Weinhold, K., Kanki, P., Carruth, L. & Schwartz, D. H. (2000) *J. Infect. Dis.* **181,** 897–903.
16. Cao, Y., Qin, L., Zhang, L., Safrit, J. & Ho, D. D. (1996) *Immunol. Lett.* **51,** 7–13.
17. Guèye-Ndiaye, A., Clark, R., Samuel, K., NDour-Sarr, A. N., Ouangre, A., Sangare, L., Mboup, S., Marlink, R., Papas, R., Child, R., *et al*. (1993) *AIDS* **7,** 481–495.
18. Sarr, A. D., Hamel, D. J., Thior, I., Kokkotou, E., Sankalé, J. L., Marlink, R. G., Coll-Seck, E. M., Essex, M. E., Siby, T., Ndoye, I., *et al*. (1998) *AIDS* **12,** 131–137.
19. Zhu, T., Mo, H., Wang, N., Nam, D. S., Cao, Y., Koup, R. A. & Ho, D. D. (1993) *Science* **261,** 1179–1181.
20. Zhang, L. Q., MacKenzie, P., Cleland, A., Holmes, E. C., Brown, A. J. L. & Simmonds, P. (1993) *J. Virol.* **67,** 3345–3356.
21. Wagner, L., Yang, O. O., Garciazepeda, E. A., Ge, Y. M., Kalams, S. A., Walker, B. D., Pasternack, M. S. & Luster, A. D. (1998) *Nature (London)* **391,** 908–911.
22. Yang, O. O., Kalams, S. A., Trocha, A., Cao, H. Y., Luster, A., Johnson, R. P. & Walker, B. D. (1997) *J. Virol.* **71,** 3120–3128.
23. Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M. & Berger, E. A. (1996) *Science* **272,** 1955–1958.
24. Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., *et al*. (1996) *Nature (London)* **381,** 661–666.
25. Doranz, B. J., Rucker, J., Yi, Y. J., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G. & Doms, R. W. (1996) *Cell* **85,** 1149–1158.
26. Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., *et al*. (1996) *Cell* **85,** 1135–1148.
27. Guillon, C., van der Ende, M. E., Boers, P. H., Gruters, R. A., Schutten, M. & Osterhaus, A. D. (1998) *J. Virol.* **72,** 6260–6263.
28. McKnight, A., Dittmar, M. T., Moniz-Periera, J., Ariyoshi, K., Reeves, J. D., Hibbitts, S., Whitby, D., Aarons, E., Proudfoot, A. E., Whittle, H., *et al*. (1998) *J. Virol.* **72,** 4065–4071.
29. Feng, Y., Broder, C., Kennedy, P. & Berger, E. A. (1996) *Science* **272,** 872–877.
30. Deng, H. K., Unutmaz, D., KewalRamani, V. N. & Littman, D. R. (1997) *Nature (London)* **388,** 296–300.
31. Rucker, J., Edinger, A. L., Sharron, M., Samson, M., Lee, B., Berson, J. F., Yi, Y., Margulies, B., Collman, R. G., Doranz, B. J., *et al*. (1997) *J. Virol.* **71,** 8999–9007.
32. Edinger, A. L., Amedee, A., Miller, K., Doranz, B. J., Endres, M., Sharron, M., Samson, M., Lu, Z.-H., Clements, J. E., Murphey-Corb, M., *et al*. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4005–4010.
33. Cocchi, F., Devico, A. L., Garzinodemo, A., Arya, S. K., Gallo, R. C. & Lusso, P. (1995) *Science* **270,** 1811–1815.
34. Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., Macdonald, M. E., Stuhlmann, H., Koup, R. A. & Landau, N. R. (1996) *Cell* **86,** 367–377.
35. Kokkotou, E., Philippon, V., Guèye-Ndiaye, A., Mboup, S., Wang, W. K., Essex, M. & Kanki, P. (1998) *J. Hum. Virol.* **1,** 469–474.
36. Walker, C. M., Moody, D. J., Stites, D. P. & Levy, J. A. (1986) *Science* **234,** 1563–1566.
37. Moriuchi, H., Moriuchi, M., Combadiere, C., Murphy, P. M. & Fauci, A. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 15341–15345.
38. Oliva, A., Kinter, A. L., Vaccarezza, M., Rubbert, A., Catanzaro, A., Moir, S., Monaco, J., Ehler, L., Mizell, S., Jackson, R., *et al*. (1998) *J. Clin. Invest.* **102,** 223–231.
39. Schmidtmayerova, H., Nottet, H., Nuovo, G., Raabe, T., Flanagan, C. R., Dubrovsky, L., Gendelman, H. E., Cerami, A., Bukrinsky, M. & Sherry, B. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 700–704.
40. Verani, A., Scarlatti, G., Comar, M., Tresoldi, E., Polo, S., Giacca, M., Lusso, P., Siccardi, A. G. & Vercelli, D. (1997) *J. Exp. Med.* **185,** 805–816.
41. Kledal, T. N., Rosenkilde, M. M., Coulin, F., Simmons, G., Johnsen, A. H., Alouani, S., Power, C. A., Luttichau, H. R., Gerstoft, J., Clapham, P. R., *et al*. (1997) *Science* **277,** 1656–1659.
42. Pleskoff, O., Treboute, C., Brelot, A., Heveker, N., Seman, M. & Alizon, M. (1997) *Science* **276,** 1874–1878.
43. Popper, S. J., Sarr, A. D., Guèye-Ndiaye, A., Mboup, S., Essex, M. E. & Kanki, P. J. (2000) *J. Virol.* **74,** 1554–1557.
44. Neoh, L. P., Akimoto, H., Kaneko, H., Hishikawa, T., Hashimoto, H., Hirose, S., Kaneko, Y., Yamamoto, N. & Sekigawa, I. (1997) *AIDS* **11,** 1062–1063.
45. Kaneko, H., Neoh, L. P., Takeda, N., Akimoto, H., Hishikawa, T., Hashimoto, H., Hirose, S., Karaki, S., Takiguchi, M., Nakauchi, H., *et al*. (1997) *J. Virol.* **71,** 8918–8922.
46. Zagury, D., Lachgar, A., Chams, V., Fall, L. S., Bernard, J., Zagury, J. F., Bizzini, B., Gringeri, A., Santagostino, E., Rappaport, J., *et al*. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3857–3861.
47. Rosenberg, E. S., Billingsley, J. M., Caliendo, A. M., Boswell, S. L., Sax, P. E., Kalams, S. A. & Walker, B. D. (1997) *Science* **278,** 1447–1450.
48. Lehner, T., Wang, Y. F., Cranage, M., Bergmeier, L. A., Mitchell, E., Tao, L., Hall, G., Dennis, M., Cook, N., Brookes, R., *et al*. (1996) *Nat. Med.* **2,** 767–775.